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Application of hot melt extrusion to enhance the dissolution and oral bioavailability of oleanolic acid

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ABSTRACT

The aim of this study was to improve the *in vitro* dissolution rate and oral bioavailability of oleanolic acid (OA), a water insoluble drug belonging to BCS class IV. Hot melt extrusion (HME) was applied to develop OA amorphous solid dispersions. The characterizations of the optimal formulation were performed by differential scanning calorimetry, X-ray powder diffraction, Fourier transform infrared spectroscopy and *in vitro* dissolution test. The *in vivo* pharmacokinetic study was conducted in rats. As a result, OA solid dispersion based on PVP VA 64 (OA-PVP) was successfully prepared. In the dissolution medium containing 0.3% SDS, OA-PVP dramatically increased the releasing rate of OA compared with the physical mixture (PM-PVP) and commercial tablet. Furthermore, OA-PVP exhibited higher AUC ($P < 0.05$) and C_{max} ($P < 0.05$) than PM-PVP and commercial tablet. The superior dissolution property and bioavailability of OA-PVP mainly attributed to the amorphous state of OA in PVP VA64 and the well dispersion caused by thermal melting and shearing. Overall, hot melt extrusion was an efficient strategy to enhance the dissolution rate and oral bioavailability of OA.

1. Introduction

Oleanolic acid (OA) is a bioactive pentacyclic triterpenoid compound widely existing in Asian herbs [1]. Its clinical pharmacology activities mainly include hepatoprotective, anti-inflammatory, antibacterial and antiulcer effect [2]. As a natural origin drug, OA owes a good application prospect due to its low toxicity. However, it exhibits low bioavailability after oral administration on account of the poor aqueous solubility (<1 μg/ml) [3] and poor permeability (P_{app} = 1.1–1.3 × 10^{-6} cm/sec in the apical-to-basolateral direction at 10

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and 20 μM) [4]. Jeong et al. reported that the absolute oral bioavailability of OA was 0.7% for oral doses of 25 and 50 mg/kg. The very low oral bioavailability of OA could be due to a poor absorption and extensive metabolic clearance [4].

Many attempts were made to improve the solubility of OA, such as solid lipid nanoparticles [5], nanosuspensions [6–8], β-cyclodextrin inclusion compounds [9], self-nanoemulsified formulations [10], phospholipid complexes [11] and solid dispersions [12,13]. However, the complicated unavailable scale-up process and the presence of organic solvents limit their application in the production process.

In recent years, hot melt extrusion (HME) has gained great interest in pharmaceutical field due to many advantages, such as scalable continuous processing, solvent-free and environmentally friendly [14,15]. In the HME process, drug and polymers are mixed thoroughly under the controlled temperature and pumped with a rotating screw through a die plate, yielding solid dispersions. To obtain a desirable product, the extrusion temperature is typically set 30–60 °C higher than the T_g (glass transition temperature) or T_m (melting temperature) of polymers, which will soften the mixture and provide a good flowability [16–18]. In addition, polymers play an important role in stabilizing the metastable solid, inhibiting crystallization [19] and maintaining supersaturation during dissolution [20,21]. Several hydrophilic polymers are developed for the HME process, for example, polyvinyl caprolactampolyvinyl acetate-polyethylene glycol graft copolymer (Soluplus®), polyvinylpyrrolidone-vinyl acetate copolymer (PVP VA64), polyethylene glycol (PEG), Eudragit® EPO, and hypromellose acetate succinate (HPMCAS). However, the miscibility of drug molecular and polymers needs to be investigated.

In the present study, we developed a HME method to improving the dissolution and oral bioavailability of OA by preparation of solid dispersions. Hydrophilic polymers, drug-to-carrier ratio and extrusion temperature were optimized. Thermal gravimetric analysis was used to detect weight loss due to degradation of OA. Differential scanning calorimetry (DSC), X-ray powder diffraction (XRPD), Fourier transform infrared (FT-IR) spectroscopy and *in vitro* dissolution were carried out to characterize the optimal formulation. Furthermore, the pharmacokinetic study was evaluated in rats.

2. Materials and methods

2.1. Materials

OA was purchased from Wuhan Dahua Pharmaceutical Co., Ltd. (Wuhan, Hubei). Soluplus® and PVP VA64 were purchased from BASF Co., Ltd. (Shanghai, China). PEG 6000 was obtained from Beijing Fengli Jingqiu Pharmaceutical Co., Ltd. (Beijing, China). Sodium dodecyl sulfate (SDS) was purchased from Tianjin Bodi Chemical Holding Co., Ltd. (Tianjin, China). H_3PO_4 was obtained from Tianjin Kemiou Chemical Reagent Co., Ltd. (Tianjin, China). Glycyrrhetinic acid was obtained from China's food and drug administration. Methanol, Methyl tert-Butyl Ether of HPLC grade was purchased from Thermo Fisher Scientific (Shanghai, China). Ammonium acetate (HPLC grade) was purchased from Dikma Technology Inc. (Beijing, China).

2.2. Preparation of hot-melt extrudates

OA was mixed with the hydrophilic carriers at a ratio of 1:10 (drug: carrier, *w/w*) to obtain the physical mixtures (PMs) by the method of increment by equal quantity. Then the PMs were extruded by a co-rotating twin-screw extruder (AK-26; Coperion Keya Co., Najing, China) at temperature of 160 °C. The screw rate was set at 20 rpm. The extrudates were collected from the die and cooled at ambient temperature. A universal high-speed smashing machine (FW400A, Zhongxingweiye instrument Co., Ltd, Beijing, China) was used to crushing the banded product into powder. After passing through an 80-mesh screen, the obtained solid dispersions powder was stored in dry dark conditions for further *in vitro* dissolution test and *in vivo* pharmacokinetic study.

2.3. Characterization of solid dispersions

2.3.1. Thermal analysis

The thermal stability of OA was determined on a thermal gravimetric analyzer (TGA-50, Shimadzu, Japan). Samples were heated from 30 to 400 °C at a rate of 10 °C/min with an empty aluminum pan as the reference. Nitrogen was used as the purge gas at a flow rate of 40 ml/min. Plots of weight versus temperature were recorded.

Differential scanning calorimetry (DSC) thermograms of the samples were conducted using a DSC1 STAR® system (Mettler Tolelo, Switzerland) equipped with a cooling system. Samples (3–5 mg) were weighted accurately and loaded in the aluminum pans. The DSC cell was purged by using nitrogen gas at flow rate of 40 ml/min. A heating rate of 20 °C/min was used to scan from 40 to 330 °C. All results were analyzed using the STARe software.

2.3.2. XRPD

XRPD analysis was performed on a D\Max-2400 X-Ray powder diffractometer (Rigaku, Japan) at ambient temperature. Monochromatic Cu-Kα radiation (λ = 1.5406 Å) was used in the 2θ angle range from 3° to 50° with a step width of 0.05°.The voltage and current of the equipment were 30 mA and 40 kV.

2.3.3. FT-IR

FT-IR spectra of samples were measured using an EQUINOX55 Fourier infrared spectrometer (Bruker, Germany) instrument. The samples were mixed with KBr using a mortar and pestle, and then compressed to prepare a disk. The spectra were recorded over a wavenumber range of 4000 cm^{-1} to 400 cm^{-1}, with a resolution of 4 cm^{-1}.

2.3.4. In vitro dissolution testing

The *in vitro* drug release from samples was tested according method 2 as described in the Chinese Pharmacopeia 2010 using a dissolution tester (ZRS-8G, Tianda Tianfa Science and Technology Ltd., Tianjin, China). Samples equivalent to 20 mg of OA were loaded into 00 capsule shells and sunk into 900 ml of deionized water containing 0.3% (*w/v*) SDS at a temperature of 37 ± 0.2 °C. The paddle rotation speed was set to 100 rpm. 5 ml of samples were withdrawn at predetermined time intervals 5, 10, 20, 30, 45, 60 and 120 min and filtered through a 0.45 μm